jection of glucagon remains unchanged in the experimental animals, the impaired response to adrenalin requires further study with larger doses of hormone before it can be established as a consequence of treatment with oxytetracycline.

Interference with the mechanism that inactivates insulin could also make for an apparent increase in sensitivity to the hormone. Insulin is rapidly inactivated in the body; its half-life in man is about 40 minutes(8). Much of the inactivation of insulin seems due to an enzyme system in the liver(9). Interference with the activity of this enzyme by oxytetracycline would cause insulin to accumulate and possibly reach a level that precipitates hypoglycemia and convulsions. It is known that oxytetracycline concentrates in the liver and that the drug interferes with the activity of enzymes—those involved in protein synthesis by staphylococci(10).

The data are limited and do not permit final conclusions. However, the induction of hypoglycemia and irreversible convulsions by oxytetracycline treatment may be of significance in the study of hormonal control of the blood sugar.

Summary. Addition of oxytetracycline to intravenous fluid used to maintain pancreatectomized dogs brings about a state of hypoglycemia with staggering and convulsions that only infrequently responds to administration of glucose. The liver glycogen of these dogs is usually normal and occasionally elevated. During the state of hypoglycemia with staggering and convulsions the rise of the blood glucose following intravenous injection of adrenalin or glucose (glucose tolerance test) is transient; the rise of the blood glucose following intravenous injection of glucagon is large and sustained.

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The Estrous Cycle and Splenic Erythropoiesis in the Mouse.* (31170)

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The normal adult mouse displays significant splenic erythropoiesis(1). Among the many experimental manipulations that further stimulate this process are several which may have in common the evocation of an inflammatory response. These include grouping of male mice which results in the wounding of subordinate animals by dominant ones (2,3), graft *vs* host reactions(4), and injection of bacterial endotoxins(5). The periodic migration of neutrophilic leukocytes into the vagina of the mouse at termination of estrus is well known and presents an opportunity for examining the possible relationship between a "physiological inflammation" and splenic erythropoiesis. Therefore, it seemed of interest to see whether or not this type of inflammation would cause increased splenic erythropoiesis.

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TABLE I. Relationship Between Estrous Cycle, Spleen and Bone Marrow in Mice (Mean ± Standard Error).

Stage based on vaginal smear	Group I Metestrus-2 and diestrus	Group II Proestrus, estrus and metestrus-1	p*
Splenic wt, mg	73 ± 2.3 (15)†	94 ± 2.7 (30)	<.01
Splenic Fe^{59} uptake, % of injected dose	3.7 ± .41 (12)	6.8 ± .34 (24)	<.01
Bone marrow‡ Fe^{59} uptake, % of injected dose	1.4 ± .56 (12)	1.2 ± .53 (24)	>.05
Splenic nucleated erythroid cells, %§	10 ± 2 (6)	23 ± 4 (6)	<.05
Bone marrow nucleated erythroid cells, %	31 ± 1.9 (9)	35 ± 2.1 (9)	>.05

* p = probability calculated from the distribution of Fisher's t.
† Figures in parentheses represent numbers of mice.
‡ 2 tibiae devoid of epiphyses.
§ Determined from spleen prints in which an attempt was made to count cells only in the red pulp and to avoid counting in areas that represented white pulp, *i.e.*, lymphoid follicles.

Methods. Adult, female CF#1 mice (Carworth Inc., New City, N. Y.) weighing 24-27 g were housed individually. Mouse pellets and water were available *ad libitum*. Vaginal smears were made each morning with a No. 0 red sable water color brush, pre-moistened with saline. These were air-dried and stained with a Wright-Giemsa mixture following which the stage of estrus was graded according to the criteria described by Snell(6). In addition, some slides were stained with Burke's modification(7) of Gram's stain.

After a preliminary survey of the results, it proved feasible to pool certain data by establishing two groups on the basis of vaginal smear cytology. Group I consisted of mice whose vaginal smears contained primarily neutrophilic leukocytes accompanied by some epithelial cells (*i.e.*, metestrus-2 and diestrus). Group II consisted of mice whose vaginal smears contained primarily cornified cells (*i.e.*, proestrus, estrus, and metestrus-1).

Approximately 0.3 μc Fe^{59} (E. R. Squibb and Sons, New Brunswick, N. J.) in 0.1 ml saline was then injected intravenously into each mouse. Preliminary studies had revealed that optimal uptake of the Fe^{59} occurred 5-10 hours after intravenous injection in both bone marrow and spleen. Therefore, mice were decapitated 5 hours after injection and radioactivity was determined in the entire spleen and both tibiae with the aid of a well-type scintillation detector. The isotope content of the whole bone was used, because the radioactivity was contained, almost entirely, in the bone marrow. In addition, touch preparations of both spleen and bone marrow were made and stained with a Wright-Giemsa mixture. From these, the percentages of nucleated erythroid cells were determined. Histologic sections of spleen were made and stained with hematoxylin and eosin.

Results. Table I summarizes the results. Spleens of Group I (estrous and metestrous-1 mice) were heavier and took up more Fe^{59} than did those of Group II (metestrous-2 and diestrous mice). Bone marrow Fe^{59} uptake did not differ significantly between the two groups. Cytologic examination of spleen prints, particularly those of the heavier spleens, revealed marked increases in the numbers of nucleated red cells, representing all stages of development including some cells in mitosis. Although splenic prints proved to be superior to sections for studying cytologic changes, the latter also showed evidence of increased splenic erythropoiesis in the red pulp. Bone marrows in both groups were markedly erythroid as evidenced by a greater than 30% nucleated erythroid count. No discernible difference between Group I and II was noted. Therefore, it appears that erythropoiesis is increased during estrus and metestrus-1 in the spleen but not in the bone marrow.

Examination of vaginal smears disclosed a rich bacterial flora composed primarily of short, plump rods which were Gram-negative. These were often adherent to cornified cells or within neutrophilic leukocytes. Bacteria were more numerous in Group II than in Group I.

Discussion. It is not clear why splenic erythropoiesis is greater near the time of

estrus. Among the variables that could play a role in this phenomenon are 1) components of the inflammatory response including endogenous breakdown products of the disintegrating vaginal epithelium, bacteria and their endotoxins, and neutrophilic leukocytes and 2) circulating hormone levels.

In view of the ability of injected bacterial endotoxins to evoke splenic erythropoiesis (6), it is possible that the bacteria found in the vagina may play some role in this regard. It would be useful to know to what extent bacteria and bacterial products enter the tissues during the various phases of the estrous cycle. Another approach to this problem might be found in the examination of germ-free mice. Such mice have been shown to display a typical leukocytic inflammatory response in the vagina following progesterone (8) and this suggests that germ-free mice may also display vaginal changes comparable to those seen in conventional mice.

Another puzzling fact is that splenic erythropoiesis was highest during estrus, a time when estrogen levels in the animal are increased, because estrogens are generally regarded as depressors of both erythropoiesis and circulating red blood cell numbers(9). A meaningful evaluation of the aforementioned variables requires more information about the time required for a given stimulus to evoke its effect. Thus it is possible that a stimulus applied during one part of a cycle may not find its full expression until some time later. Studies currently in progress to determine the effects of ovariectomy, injected hormones and pregnancy upon splenic erythropoiesis may help to elucidate some of the questions that have been raised.

Summary. Ferrokinetic and cytologic studies in female mice reveal that splenic erythropoiesis is heightened during proestrus, estrus, and metestrus-1 as compared to metestrus-2 and diestrus.

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A Possible Explanation for Decreased Tryptophan Pyrrolase Activity In Homogenates of Liver from Endotoxemic Mice.* (31171)

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The decrease in activity of tryptophan pyrrolase in homogenates of liver from endotoxin poisoned mice(1) is considered to have special significance since it implies an initial block in the pathway leading to the formation of pyridine nucleotides *in vivo*. Guided by the hypothesis that impaired biosynthesis of essential corequirements for the production of energy could represent a primary biochemical lesion in endotoxin poisoning, related investigations reported to date have been attempts to correlate tryptophan pyrrolase activity to survival of poisoned mice during various experimental treatments, *e.g.*, Berry and Smythe(2) and Berry(3). Eaves and Berry(4) subsequently reported that plasma from mice injected with heat-killed *Salmonella typhimurium* inhibited tryptophan pyrrolase activity in whole homogenates of

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